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## Full Length Article

# Clinical, hemato-biochemical alterations and oxidant–antioxidant biomarkers in *Babesia*-infected calves

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**Abstract** *Babesia* is one of the main causes of anemia in cattle, a lot of elucidations have been suggested to explain its pathogenesis. This study was designed to investigate clinical, hemato-biochemical and oxidant/antioxidant status and its relation with the resultant anemia in *Babesia*-infected calves. Seventeen (17) native breed calves were involved in this study, clinical signs and microscopic findings were recorded, also blood samples were taken to investigate hematologic changes, serum biochemical variations and oxidative stress biomarkers. The most commonly observed clinical signs were fever, emaciation, depression, icterus and hemoglobinuria. Significant reduction in PCV, HB, RBCs, MCHC, Total protein, and albumin along with significant increase in MCV, WBCs, monocytes and BUN were the most consistent hemato-biochemical changes. Oxidant/antioxidant and trace mineral assessment showed significant reduction in Superoxide dismutase "SOD", Glutathione peroxidase "GPx", Zn, Cu along with significant increase in malondialdehyde (MDA) activities. In the current investigation, oxidant/antioxidant imbalance along with the synchronized alterations in antioxidant trace minerals was detected in *Babesia*-infected calves. These findings support notion that *Babesia* infection associated with oxidative stress and this process may be linked to the resultant anemia.

## 1. Introduction

Livestock in Egypt plays a considerable role in country economy. Cattle is one of the main livestock population raised in Egypt, according to FAO [1], cattle population in 2009 ranged from 3.5 to 5 million head.

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Cattle industry can be plagued with abundant diseases, among which tick-borne ailments are of considerable importance. These diseases have a negative influence on animal health and subsequently the economy [2]. Bovine babesiosis is an important tick-borne ailment all over the world but mostly in subtropical and tropical territories [3]. *Babesia* is an intraerthrocytic hemoprotozoan affecting animal erythrocytes [4]. In Egypt, bovine babesiosis are caused by *B. bovis* and *B. bigemina* [5,6].

Babesiosis common presentation is pyrexia, anemia, icterus, emaciation, inappetence and hemoglobinuria, in pregnant cattle, diarrhea and even abortion may occur [7,8]. Bovine babesiosis was known to cause anemia, the type of anemia usually hemolytic, though, ranged from hypochromic to normochromic, this change is associated with reduction in erythrogram parameters and alterations in serum biochemical values [3]. The microscopic examination of blood smear considered the most applicable and the cheapest method for diagnosis, presence of the protozoan inside red cells considered confirmative especially during acute stage of the disease, this ability decrease sharply in other situations like carrier animals [9,10].

Oxidative stress is a disproportion between scavenging mechanism and radical generating mechanism [11]. The erythrocyte peroxidation occurs in hemo-protozoan infection may be correlated to disease pathogen [12]. Free radicals can harm tissue and cells, when Reactive oxygen species "ROS" exceed the ability of antioxidant system, oxidative process ensues [13]. Recently in blood parasites investigations, strong accumulated evidences were found to link the resultant anemia with oxidative damage due to lipid peroxidation of red cells [12]. Anti-oxidant elements such as SOD and GPx are vital in counteract ROS damage [11].

The purpose of this study is to describe the clinical and laboratory alterations in *Babesia*-infected calves with special emphasis on oxidant-antioxidant status associated with this disease.

## 2. Materials and methods

### 2.1. Animals

Seventeen (17) clinically-ill native breed male calves aged 1 year, located at Alexandria-Cairo desert road, Egypt were involved in this study. Criteria of inclusion were presence of tick infestation, clinical signs and positive microscopic blood smear. Giemsa-stained thin blood films were inspected under the light microscope for identification of intraerythrocytic stages of the hemoparasite [14]. Control calves ($n = 6$) from same farm with the same age and sex were involved. Calves were thoroughly clinically examined, fecal samples were taken and blood smears from each control calf was examined microscopically to ensure they are parasitological free. The criteria for inclusion in control group were also depended on no former or existing history of tick exposure, and absence of clinical signs.

Each calf was subjected to comprehensive clinical examination and clinical signs were recorded.

### 2.2. Blood sampling and microscopic detection

Blood samples were collected from jugular vein in EDTA-containing tubes and plain tube to separate serum samples;

the blood in plain tubes were preserved in slanted spot for approximately 2 h and then refrigerated at 4 °C overnight for serum separation. Clinical hematology was performed within 2 h after the sample collection. Three Giemsa stained blood films from each animal were examined under oil-immersion lens, to confirm the *Babesia* infection.

### 2.3. Hematologic investigations

For hematological analysis, Haemo-cytometric method was used to determine erythrocyte and leukocyte counts. Hemoglobin was estimated using Drabkin colorimetric method. Hematocrit values were evaluated by micro-hematocrit centrifugation. Differential leukocyte counts were determined from smears stained by the Giemsa method. The erythrocyte indices of MCV and MCHC values were also estimated by appropriate formulas.

### 2.4. Estimation of oxidant-antioxidant status

EDTA whole blood samples were used to formulate erythrocytes lysates for estimation levels of glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes using respective test kits (Bio-Diagnostic Company-Egypt). While resultant plasma was used to estimate catalase using respective test kit (Bio-Diagnostic Company-Egypt). Estimation of these parameters was done manually using spectrophotometer (APEL, PD-303S, Japan) according to manufacturer instructions.

### 2.5. Estimation of serum biochemical parameters

Serum samples were used to determine total protein, albumin, cholesterol, triglycerides, BUN, Malondialdehyde (MDA), zinc (Zn) and copper (Cu) (Bio-Diagnostic Company-Egypt, Spectrum Diagnostic, and Egypt). Estimation of these parameters was done manually using spectrophotometer (APEL, PD-303S, Japan) according to manufacturer instructions.

### 2.6. Statistical analysis

Results of diseased calves were compared to control data, calculation of mean $\pm$ SE, and data comparison using student t-test (STATISTICA for Windows, version 5.1., StatSoft, Inc.). $P \leqslant 0.05$ considered significant.

## 3. Results

The most consistent clinical signs recorded in diseased calves were fever, tachypnea, decreased weight, anorexia, inappetence and depression; icterus and hemoglobinuria were also observed. Tick infestation was detected on affected calves.

The examination of blood films revealed the presence of pyriform merozoites of *B. bigemina* at an acute angle inside the erythrocytes, and only the calves that showed positive on microscopic examination were included in this study.

Hematologic variations in *Babesia* infected calves are shown (Table 1 and Fig. 1). Significant decrease ($P \leqslant 0.05$) in RBCs, HB, PCV, MCHC, platelet count along with significant increase in MCV was observed in diseased calves

**Table 1** Hematologic evaluation of erythrogram and leukogram of diseased calves compared with the apparently healthy calves.

| Parameter | Control Calves (A) | Diseased Calves (B) | P value |
|---|---|---|---|
| RBCs($10^6 \times mm^3$) | 8.59 ± 0.51 | 6.026 ± 0.224 | 0.001 |
| PCV (%) | 32 ± 1.140 | 25.892 ± 1.099 | 0.004 |
| HB (g/dl) | 11.05 ± 0.31 | 9.56 ± 0.366 | 0.021 |
| WBCs ($10^3 \times mm^3$) | 10.640 ± 0.928 | 24.630 ± 0.566 | 0.0252 |
| Neutrophils (%) | 41.40 ± 5.01 | 53.67 ± 5.61 | 0.173 |
| Lymphocytes (%) | 52.60 ± 4.2 | 36.50 ± 4.75 | 0.049 |
| Eosinophils (%) | 2.50 ± 0.5 | 1.75 ± 0.37 | 0.259 |
| Monocytes (%) | 6.00 ± 0.71 | 11.10 ± 1.23 | 0.028 |
| MCV (%) | 38 ± 0.860 | 42.80 ± 0.77 | 0.016 |
| MCHC ($\mu\mu^3$) | 36.70 ± 0.33 | 35 ± 0.32 | 0.006 |
| Platelets ($10^3 \times mm^3$) | 450.200 ± 90.481 | 290.777 ± 21.195 | 0.0451 |
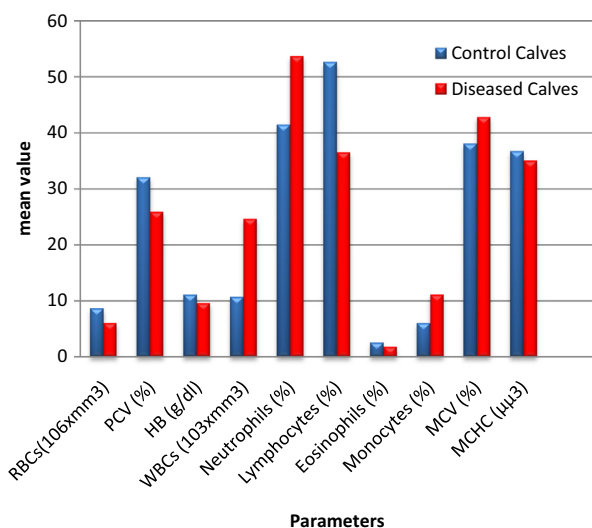


**Fig. 1** Hematologic alterations in diseased group compared to control group.

**Table 2** Serum biochemical evaluation in diseased calves compared with the apparently healthy calves.

| Parameter | Control Calves (A) | Diseased Calves (B) | P value |
|---|---|---|---|
| Total Protein (g/dl) | 6.97 ± 0.21 | 5.286 ± 0.5 | 0.001 |
| Albumin (g/dl) | 3.65 ± 0.155 | 2.71 ± 0.20 | 0.013 |
| Cholesterol (mg/dl) | 70.75 ± 7.94 | 65.36 ± 7.11 | 0.652 |
| Triglycerides (mg/dl) | 10.0880 ± 1.112 | 8.374 ± 0.9570 | 0.2857 |
| BUN (mg/dl) | 10.0600 ± 1.045 | 19.460 ± 3.4958 | 0.0424 |

compared to control data, while platelet count showed decrease in diseased calves compared to control data. In leucogram, significant increase ($P \leqslant 0.05$) in WBCs and monocytes was observed in diseased calves while lymphocyte was significantly decreased. Neutrophils in diseased calves although numerically higher than control data but this elevation was considered to be not quietly statistically significant.

Serum biochemical changes are recorded (Table 2). Significant decrease ($P \leqslant 0.05$) in total protein and albumin along with significant increase in BUN was recorded in diseased calves, where no statistical changes were observed in cholesterol and triglycerides.

Results of oxidative stress biomarkers are shown (Table 3 and Fig. 2). Significant increase ($P \leqslant 0.05$) in MDA accompanied by significant decrease in SOD, GPx, Zn and Cu in diseased calves compared to control data was reported, whereas catalase showed increase in diseased calves though this increase considered not statistically significant.

## 4. Discussion

Tick-borne diseases have a negative effect on livestock health [11], and responsible for major economic losses.

In this study, *Babesia* infection was confirmed via light microscopy examination. Presence of pear shape piroplasms inside RBCs is confirmative of diagnosis especially in acute stages of the disease [9].

Fever, emaciation, tachypnea, anorexia, depression, icterus and hemoglobinuria were observed, similar manifestations were reported [15,16]. The major modus operandi by which the protozoan operates is hemolysis which leads to anemia and subsequent inflammatory process in kidney and liver [7]. It has been speculated that in the face of every clinical condition, there are tens of latent undiscovered conditions [17]. Correlation between *Babesia* and release of "pharmacologic-active mediators" was found [18] that stimulates certain mediators causing circulatory failure with coma and death eventually [19].

Significant decrease in RBCs, HB, PCV, MCHC and platelets values along with significant increase in MCV was observed in diseased calves, the erythrogram results advocates the presence of macrocytic hypochromic anemia.

Anemia is a feature of *Babesia*; the parasite is capable of destroying RBCs when it exists [19]. In *B. bigemina*; parasitemia is adept to cause a major alteration in total RBCs osmotic fragility even intact unaffected RBCs, these red cells become easily destroyed creating hemolysis [20]. In other animal models, auto-antibodies against circulating red cells have been suggested [21]. The antibody-coated red cells are attacked by macrophages and complements bound to membrane via Ag–Ab reaction creating hemolysis [22].

The resultant decrease in platelets is attributed to decrease the production or increase destruction [23]. Thrombocytopenia is usually correlated with immune-mediated process and hemolysis [24].

Leukocytosis and monocytosis are observed in clinically infected calves, and these findings were observed in *Babesia* infected animals [25,26]. The ability of the protozoan to trigger lymphoid system linked to increase in WBCs count during infection [27]. Monocytosis is predictable in hemolytic diseases [28].

Significant decrease in Total protein and albumin with elevated BUN levels was observed in diseased calves. *Babesia* can cause disruption in liver function that leads to decrease albumin synthesis and consequently affect total protein levels [29,30]. *Babesia* can cause degeneration and necrosis in kidney convoluted tubules, consequently a rise in BUN is expected [7].

In recent years, there were cumulative evidences suggest the pathogenesis of anemia in babesiosis is correlated with lipid

**Table 3**  Oxidant/antioxidant changes in diseased calves compared with the apparently healthy calves.

| Parameters | | Control Calves (A) | Diseased Calves (B) | P value |
|---|---|---|---|---|
| Enzymatic anti-oxidant | SOD (u/ml) | 247.795 ± 20.171 | 141 ± 12.20 | 0.0013 |
| | GPx (mu/ml) | 266.196 ± 31.5 | 166.40 ± 21.870 | 0.027 |
| | Catalase (u/l) | 297.6667 ± 19.35 | 321.298 ± 47.116 | 0.775 |
| Anti-oxidant-Trace Element | Cu (umol/l) | 16.837 ± 0.489 | 12.65 ± 0.74 | 0.004 |
| | Zn (ug/dl) | 27.901 ± 3.23 | 12.497 ± 1.574 | 0.006 |
| Oxidant | MDA (nmol/ml) | 1.566 ± 0.173 | 4.664 ± 0.415 | 0.004 |
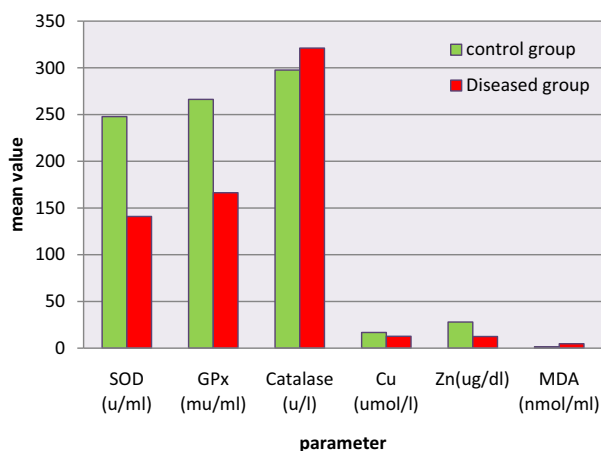


**Fig. 2**  Oxidant/antioxidant alterations in *Babesia* infected calves.

peroxidation and oxidative process [31]. Our data showed increase in oxidant system along with decrease in antioxidant system. Significant rise in MDA was observed, MDA is a profound index of lipid peroxidation process [32]; this increase leads to buildup of oxidative ions in RBCs causing its lyses [33].

RBCs membrane structure is rich in polyunsaturated fatty acids, *Babesia* is capable of making the red cell membrane highly prone to per oxidative impairment [11,34,35]. It appears that *Babesia* has a great influence on the oxidant/antioxidant system resulting in ROS production in substantial quantities among which is MDA, which subsequently overload the antioxidant system and cause direct damage to red cells [11,34,35].

The increase in oxidant markers was coupled with exhaustion of antioxidant markers (GPx and SOD), important indicators of oxidative stress. GPx is an important antioxidant enzyme that is widely known to metabolize lipid peroxidation products [36]. GPx plays a part in catabolism of ROS, it has been suggested that "peroxide-mediated deactivation" of GPx can occur [37]. The role GPx played in protection against toxic effect of ROS can explain the decrease in its activity in infection.

SOD an antioxidant enzyme known for its ability to scavenge toxins from body [38]. In this study SOD activity showed a significant decrease compared to control data, SOD plays an integral part in RBCs protection system against oxidative damage [39]. Presence of hemo-protozoan inside RBCs considerably affects the key anti-oxidant system of red cells, leading

to a state in which red cells bump into oxidant mediators' assault which eventually will cause cell injury and hemolysis [40]. It appears the protozoan- prompted modulation of anti-oxidant enzymes cause a speed up in RBCs removal via phagocytosis [41].

In contrary to SOD and GPx pattern, Catalase showed non-significant increase in diseased calves. Similar results were found in *T. annulate* infected cows and *Thelieria*-infected sheep [42,43]. Catalase is accountable for $H_2O_2$ removal and thus it provides protection against damaging effect of $H_2O_2$ leading to rise RBCs lifetime, so the increase in its activity may be contributed to the indirect compensatory reaction to oxidant encounter [43].

The enzymatic antioxidant mechanism activities rely primary on certain trace minerals among them Zinc and Copper [44]. Cu and Zn play an important role in body antioxidant mechanism by preventing radical-induced injury [40]. Zn can antagonize the redox active agents and decreases cellular injury, though the properties of zinc as antioxidant have been already established, the mechanism is unclear [45]. Copper has been recorded to decrease in bovine babesiosis [11,46]. Cu–Zn SOD catalyzing the alteration of $O_2^-$ to $H_2O_2$ [41]. Copper can perform as both a pro-oxidant and an antioxidant, Cu removes free radicals and helps in preventing them from exerting some of their damaging effect [47].

## 5. Conclusion

In the current investigation, oxidant/antioxidant imbalance along with the synchronized alterations in antioxidant trace minerals was detected in *Babesia*-infected calves. These findings support notion that *Babesia* infection associated with oxidative stress and this process may be linked to the resultant anemia.

### Declaration

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